REMARKS

Claims 35 to 53 are presently pending, with claim 37 withdrawn from consideration as allegedly directed to a non-elected invention. New claims 54 to 100 have been added herein. Thus, claims 35, 36 and 38 to 94 are presently under examination.

Regarding the amendment to the specification

The specification has been amended at page 40 to correct a typographical error. In particular, the sentence at page 40, lines 10-11, has been amended to indicate that a scissile bond cleaved by BoNT/A can be, for example, "Gln-Arg" rather than "Gln-Ala." The amendment to page 40 is supported throughout the specification as filed, for example, at page 39, lines 16-20, which indicates that BoNT/A substrates can include at least six consecutive residues of SNAP-25 including "Gln-Arg," and at page 31, Table 1, line 8, which indicates that the SNAP-25 bond cleaved by BoNT/A is "Gln-Arg." In sum, the amendment to the specification is supported by the application as originally filed and does not add new matter. Accordingly, the Examiner is respectfully requested to enter the amendment to the specification.

Regarding the claim amendments and new claims

Original claim 35 has been amended herein to recite specific lengths of BoNT/A substrate. The amendment to claim 35 is supported throughout the specification as filed, for example, at page 40, lines 13-15, and page 115, lines 3-15, which discloses specific lengths of BoNT/A substrate. New claims 54 to 59 depend from claim 35 and separately recite specific lengths of BoNT/A substrate.

New claims 60 to 78 have been added herein. These new claims are supported by the specification and claims as originally filed and do not add new matter. In particular, new claim 60 is supported by original claims 4 and 9 and throughout the specification, for example, at page 4, lines 1-20, which discloses BoNT/A substrates having at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2) including Gln_{197}-Arg_{198} or a peptidomimetic thereof, and at page 7, line 21, to page 8, line 2, which discloses BoNT/E substrates having at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2) including Arg_{180}-Ile_{181} or a peptidomimetic thereof. Similarly, new claim 61 is supported by original claim 4 and throughout the specification, for example, at page 4,

lines 1-20, and new claim 62 is supported by original claim 9 and throughout the specification, for example, at page 7, line 21, to page 8, line 2, described above. New claims 63 to 78 additionally are supported, for example, by original claims 38 to 53, respectively.

New claims 79 to 100 have been added herein. These new claims are supported by the specification and claims as originally filed and do not add new matter. In particular, new claims 79 to 82 are supported, for example, by original claim 35 and at page 86, line 12, to page 87, line 8, which describes the use of genetically encoded donor fluorophore or acceptor. New claims 83 to 100 are supported, for example, by original claims 36 to 53.

As set forth above, each of the amendments and new claims is supported by the specification or claims as originally filed and does not add new matter. The Examiner is respectfully requested to enter the claim amendments and new claims.

Regarding the double patenting rejection

The provisional rejection of claims 35, 36, 38, 39, 41, 42, 44, 45, 47, 48 and 53 under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 61 to 63, 67, 71 to 74, and 76 to 82 of U.S. application Serial No. 10/261,161 is respectfully traversed. While this provisional rejection is traversed, Applicants respectfully defer responding to the rejection until allowable subject matter has been indicated.

Regarding the rejection under 35 U.S.C. §103

Applicants respectfully traverse the rejection of claims 35, 36 and 38 to 53 under 35 U.S.C. §103(a) as allegedly obvious over Schmidt and Bostian (U.S. Patent No. 5,965,699) in view of Clegg, Curr. Opin. in Biotech. 6:103-110 (1995).

Applicants maintain that the claimed methods are unobvious over Schmidt and Bostian in view of Clegg. The primary reference by Schmidt and Bostian appears to describe a method of labeling BoNT/A cleavage product with fluorescamine for quantitation of BoNT/A but does not teach fluorescence resonance energy transfer assays for determining BoNT/A or BoNT/E protease activity. The secondary reference by Clegg is cited as reporting the use of fluorescence resonance energy transfer assays to study enzymes. In regard to combining these references, the

Examiner has acknowledged that obviousness "can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in art." As in the prior Office Action, the Examiner continues to assert that the motivation to modify the assay of Schmidt and Bostian by incorporating FRET comes from the teaching of Clegg that

> 'The benefits of fluorescence resonance energy transfer are becoming increasingly evident to researchers who require measurements with high sensitivity, specificity, noninvasiveness, rapidity and relative simplicity' (Office Action at page 5, first paragraph).

Applicants dispute, however, that Clegg's motivation to produce assays with "high sensitivity, specificity, noninvasiveness, rapidity and relative simplicity" would have been sufficient to motivate one skilled in the art to modify the fluorescamine-based assay of Schmidt and Bostian. Specifically, each of the five advantages of FRET emphasized by Clegg were already present in the assay of Schmidt and Bostian. Firstly, as Applicants have previously argued in regard to assay sensitivity, Schmidt and Bostian indicate at column 2, lines 39-40, that their "[a]ssays are very sensitive (2 to 5 nanograms per milliliter toxin can be detected)." Secondly, in regard to the specificity of a clostridial toxin assay, assay specificity is understood to be determined by the recognition sequence of the substrate and is not expected to be improved by incorporation of the donor fluorophores and acceptors required for a FRET assay. Thirdly, the assay of Schmidt and Bostian, which is performed *in vitro,* is non-invasive. Thus, one skilled in the art would not have been motivated to modify the substrate of Schmidt and Bostian in order to produce a non-invasive assay. Fourthly and fifthly, the assay of Schmidt and Bostian is reported to be rapid and relatively simple. Schmidt and Bostian themselves state that their assay can be performed "in one hour or less since no separation of hydrolysis products is needed (column 2, lines 24-26; column 2, lines 39-44; and column 4, lines 23-26). In sum, one skilled in the art understands that the Schmidt and Bostian fluorescamine-based assay, which does not utilize FRET, already had high sensitivity, specificity, noninvasiveness, rapidity and relative simplicity. Thus, such general benefits of FRET as provided by Clegg would not have been sufficient to motivate one skilled in the art to modify the Schmidt and Bostian assay. Applicants respectfully request that the Examiner address Applicants' argument that Clegg's emphasis on

assays of "high sensitivity, specificity, noninvasiveness, rapidity and relative simplicity" would not have been sufficient to motivate one skilled in the art to combine the cited references in order to produce the claimed invention.

With respect to the "label-based assay" and use of "fluorescamine" described in Schmidt and Bostian, Applicants contend that the description of a label-based assay and use of fluorescamine in this reference further corroborates Applicants' position that there was no motivation to combine the description in Schmidt and Bostian with the teachings in Clegg. The assay described by Schmidt and Bostian "is based on the well-known fact that proteolytic cleavage of peptides or proteins results in production of new free amino groups" (column 3, lines 24-26, emphasis added). The reference further discloses that "any reagent that reacts with amino groups could be used as a label for this determination" (column 3, lines 35-36, emphasis added). Fluorescamine is used because it is not fluorescent but becomes "intensely fluorescent" after it reacts with amines (column 4, lines 13-16). Thus, the basis of the botulinum toxin assay described in Schmidt and Bostian is to label the free amino group generated by proteolytic cleavage with a molecule that becomes fluorescent upon reaction with the free amino group. In contrast, the relevance of the cleavage reaction in a FRET assay, as disclosed in the specification and described in Clegg, is to allow diffusion of the quenching donor-acceptor pair to generate a fluorescent signal rather than the generation of a free amino group for subsequent labeling with a fluorescent label, as in the assay of Schmidt and Bostian.

Furthermore, even if, *arguendo*, the assay of Schmidt and Bostian was modified to include a FRET donor and acceptor, addition of the free amino group labeling reagent of Schmidt and Bostian could, in fact, interfere with the FRET assay since an additional fluorescent agent would be added to the substrate fragment containing the fluorescent donor or acceptor being measured, depending on the position of the donor and acceptor relative to the cleavage site. Clearly there would have been no motivation to modify the assay of Schmidt and Bostian, which is based on the generation of a free amino group for the subsequent incorporation of a fluorescent label, to incorporate a donor fluorophore and acceptor into the substrate prior to the cleavage reaction. The lack of motivation to combine the cited references is further substantiated by the fact that, even though the Clegg review article, which appeared in the well known "Current Opinion" series of review publications, was available a year and a half before the filing

date of Schmidt and Bostian (filing date November 6, 1996)(see Exhibit A, PubMed printout showing publication of Clegg in February 1995), the Schmidt and Bostian reference provides no teaching or suggestion for modifying the assay to incorporate FRET. Furthermore, the alleged motivation provided by Clegg (Office Action, page 5) to modify the assay of Schmidt and Bostian to incorporate FRET would require a complete change in the type of assay to be performed, an assay based on labeling of a free amino group resulting from proteolytic cleavage, or would require combining the assay of Schmidt and Bostian with a FRET assay as described in Clegg that could produce products more difficult to assay due to the incorporation of two fluorescent molecules. Clearly the skilled artisan would have had no such motivation, as discussed above.

In sum, Applicants respectfully request that the Examiner reconsider and remove the rejection of claims 35, 36 and 38 to 53 under 35 U.S.C. § 103.

Regarding the rejection under 35 U.S.C. §102(e)

The rejection of claims 35, 36 and 38 to 47 under 35 U.S.C. §102(e) as allegedly anticipated by Schmidt et al., U.S. Patent No. 6,762,280 ("the '280 patent"), respectfully is traversed.

The Office Action indicates that the cited '280 patent describes substrates for clostridial toxins, including modified peptides or proteins useful as FRET substrates (abstract and column 4). Specifically, the '280 patent allegedly reports FRET clostridial toxin substrates in which a fluorescent group and a molecule that quenches fluorescence are positioned on either side of a cleavage site (columns 5 and 7). The Office Action further asserts that the '280 patent reports cleavage of SNAP-25 by BoNT/A and that such protease activity could form the basis for a practical assay of BoNT/A.

Applicants respectfully note that the '280 patent is cited as a §102(e) reference based on the filing date of the priority provisional application Serial No. 60/235,050, filed on September 25, 2000. The actual filing date of the application which matured into the '280 patent, September 25, 2001, does not predate the subject application, which has a filing date of August 28, 2001. Thus, Applicants respectfully remind the Examiner that the '280 patent is proper §102(e) prior art only for

subject matter disclosed in the priority provisional application Serial No. 60/235,050, filed on September 25, 2000. For the Examiner's convenience, a copy of priority provisional application Serial No. 60/235,050 is attached as Exhibit B.

Applicants submit that the methods of claims 35, 36 and 38 to 47 are novel over the '280 patent. As amended herein, independent claim 35 is drawn to a method of determining protease activity of BoNT/A or BoNT/E using a substrate which is cleaved by BoNT/A or BoNT/E, wherein the BoNT/A substrate has a length of amino acids selected from the group consisting of 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 69 amino acids and 72 amino acids. The remaining rejected claims all depend from claim 35. In contrast to the claimed methods, the priority provisional application to the '280 patent, attached hereto as Exhibit B, at best describes determining botulinum activity using a single short BoNT/A synthetic peptide substrate in which a fluorescent group and a molecule that quenches fluorescence are positioned on either side of a cleavage site. The sequence of the synthetic BoNT/A peptide substrate is set forth at page 1 of the priority provisional application Serial No. 60/235,050, as follows:

N(alpha)-acetyl-S N R T R I D X A N **Q R** A Z R M L (amide), where "X" is N(epsilon)-(2,4-dinitrophenyl)-lysine and "Z" is S-(fluoresceinyl)-cysteine.

Applicants assert that the priority provisional application Serial No. 60/235,050 does not teach the claimed methods, in which the BoNT/A substrate has a length of amino acids selected from the group consisting of 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 69 amino acids and 72 amino acids. Thus, the cited 102(e) reference cannot anticipate the invention.

Accordingly, Applicants respectfully request that the Examiner reconsider and remove the rejection of claims 35, 36 and 38 to 47 as allegedly anticipated under 35 U.S.C. § 102(e) by Schmidt et al., U.S. Patent No. 6,762,280.

Regarding new claims 60 to 78

New claims 60 to 78 are directed to methods of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E) by (a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate which contains (i) a donor fluorophore; (ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of

said donor fluorophore; and (iii) a BoNT/A recognition sequence containing a cleavage site, where the BoNT/A recognition sequence has at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2) and includes Gln_{197}-Arg_{198} or a peptidomimetic thereof, or a BoNT/E recognition sequence containing a cleavage site, where the BoNT/E recognition sequence has at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2) and includes Arg_{180}-Ile_{181} or a peptidomimetic thereof, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor; (b) exciting the donor fluorophore; and (c) determining resonance energy transfer of the treated substrate relative to a control substrate, where a difference in resonance energy transfer of the treated substrate as compared to the control substrate is indicative of BoNT/A or BoNT/E protease activity. Thus, where a BoNT/A recognition sequence is included in the substrate recited in new claims 60 to 78, the BoNT/A recognition sequence has at least six consecutive residues of the wild type human SNAP-25 sequence, with these six residues including, in part, the Gln_{197}-Arg_{198} BoNT/A cleavage site. Similarly, where a BoNT/E recognition sequence is included in the substrate recited in new claims 60 to 78, the BoNT/E recognition sequence has at least six consecutive residues of the wild type human SNAP-25 sequence, with these six residues including, in part, the Arg_{180}-Ile_{181} BoNT/E cleavage site.

In contrast to the methods of new claims 60 to 78, Schmidt et al. do not describe methods which rely on a substrate having at least six consecutive residues of the human wild type SNAP-25 sequence (SEQ ID NO: 2) and including the Gln_{197}-Arg_{198} BoNT/A cleavage site or the Arg_{180}-Ile_{181} BoNT/E cleavage site. At best, Schmidt et al. describe a BoNT/A substrate containing fewer than six consecutive residues of human SNAP-25 (SEQ ID NO: 2) and including the Gln_{197}-Arg_{198} BoNT/A cleavage site. As can be readily appreciated by review of Table 1 below, the Schmidt et al. substrate differs from the wild type human SNAP-25 (SEQ ID NO: 2) sequence in the region of six consecutive residues including the Gln_{197}-Arg_{198} cleavage site. In particular, in the BoNT/A substrate of Schmidt et al., a region of six consecutive residues including the Gln_{197}-Arg_{198} cleavage site will necessarily contain either residue "X," a modified lysine residue substituted for the wild type human SNAP-25 residue glutamate, or the residue "Z," a modified cysteine residue substituted for the wild type human SNAP-25 residue threonine. Failing to describe a BoNT/A or BoNT/E substrate which contains at least six consecutive residues of human SNAP-25 (SEQ ID NO: 2)

including the Gln_{197}-Arg_{198} BoNT/A cleavage site or the Arg_{180}-Ile_{181} BoNT/E cleavage site, Schmidt et al. cannot anticipate the invention of new claims 60 to 78.

Table 1

Comparison of the wild type human SNAP-25 sequence surrounding the BoNT/A cleavage site with the synthetic BoNT/A peptide 1 of Schmidt et al.

Residue position	193	194	195	196	**197**	**198**	199	200	201	202
human SNAP-25 (SEQ ID NO: 2)	Asp	Glu	Ala	Asn	**Gln**	**Arg***	Ala	Thr	Lys	Met
Schmidt et al. peptide 1	Asp	X	Ala	Asn	**Gln**	**Arg***	Ala	Z	Arg	Met

where X = N(epsilon)-(2,4-dinitrophenyl)-lysine and Z = S-(fluoresceinyl)-cysteine
* cleavage site shown in bold

Regarding New Claims 79 to 100

New claims 79 to 100 are directed to methods of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E) by (a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate which contains (i) a donor fluorophore; (ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and (iii) a BoNT/A or BoNT/E recognition sequence comprising a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor, where the donor fluorophore or the acceptor is genetically encoded; (b) exciting the donor fluorophore; and (c) determining resonance energy transfer of the treated substrate relative to a control substrate, where a difference in resonance energy transfer of the treated substrate as compared to the control substrate is indicative of BoNT/A or BoNT/E protease activity.

In contrast to the methods of new claims 79 to 100, Schmidt et al. does not teach or suggest the use of a genetically encoded fluorophore or acceptor. At best, Schmidt et al. describes the chemical compounds "fluorescein and dinitrophenyllysine" as examples of "signal and quench

moeities [sic]" (Exhibit B, page 2, first paragraph). Accordingly, Schmidt et al. cannot anticipate the invention of new claims 79 to 100.

CONCLUSION

The Examiner is respectfully requested to consider the above remarks. Should he have any questions, the Examiner is invited to call the undersigned agent or Cathryn Campbell.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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1: Curr Opin Biotechnol. 1995 Feb;6(1):103-10. Related Articles, L

Fluorescence resonance energy transfer.

Clegg RM.

Max Planck Institute for Biophysical Chemistry, Gottingen, Germany.

In the past year, a number of studies have demonstrated the utility of fluorescence resonance energy transfer as a technique for probing complex intermolecular interactions and for determining the spatial extension and geometrical characteristics of multicomponent structures composed of divers molecular constituents, such as proteins, lipids, carbohydrates, nucleic acids, and even cells with viruses. The benefits of fluorescence resonance energy transfer are becoming increasingly evident to researchers who require measurements with high sensitivity, specificity, non-invasiveness, rapidity, a relative simplicity.

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- Review, Tutorial

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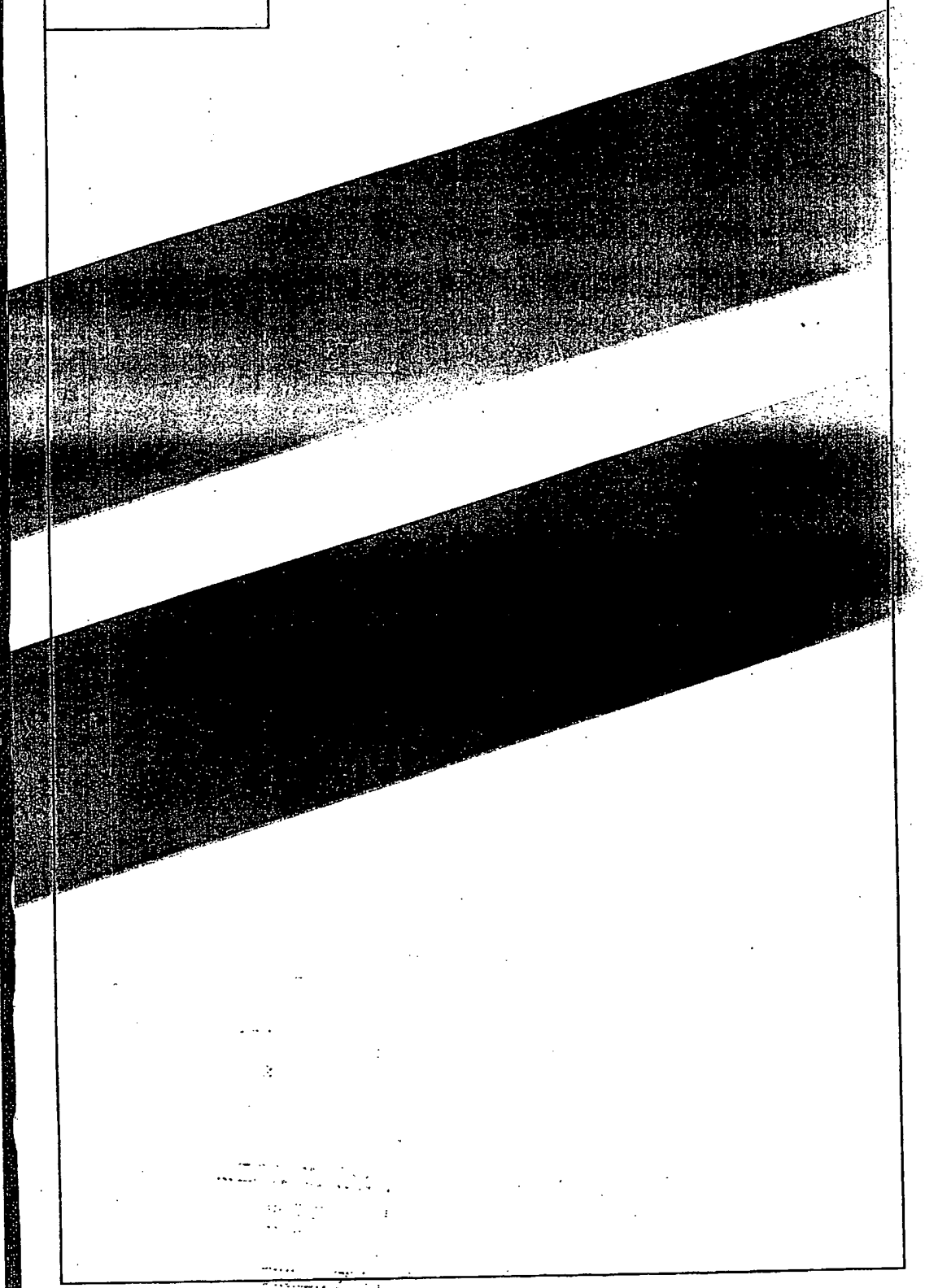
ISSUE CLASSIFICATION	Class	Subclass

[redacted]

Joan DLW
P.G.

M.H

PROVISIONAL APPLICATION NUMBER



Form PTO-1625
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EXHIBIT B
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PATENT APPLICATION

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PATENT AND TRADEMARK OFFICE
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*U.S. GPO: 1988-468-082/19144

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60/235,050	09/25/2000 RULE –	–	–	003/204/SAP RIID 00-38X

APPLICANTS
James J. Schmidt, Mt. Airy, MD ;
Robert G. Stafford, Ransom, WV ;

** CONTINUING DATA **********

** FOREIGN APPLICATIONS **********

IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 12/11/2000

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☐ no 35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance Verified and Acknowledged Examiner's Signature Initials	MD	2	–	–

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TITLE
Substrates for high-throughput assays of clostridial neurotoxin proteolytic activities

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Title: Substrates for High-Throughput Assays of Clostridial Neurotoxin Proteolytic Activities.

The invention consists of two types of compounds: (I), modified peptides or proteins that can serve as quenched-signal substrates in assays for the proteolytic activities of clostridial neurotoxins; and (II), modified peptides or proteins that can serve as immobilized substrates in assays for the proteolytic activities of clostridial neurotoxins. The assays are called "high-throughput" because lengthy processing steps such as centrifugation, solid-phase extraction, or chromatography are not required. Therefore, the assays can be readily adapted for use in automated or robotic systems. The term "clostridial neurotoxins" refers to the seven serotypes of neurotoxin (types A through G, inclusive) produced by *Clostridium botulinum* , and to tetanus toxin, produced by *Clostridium tetani*.

Type (I) compounds claimed:

Claim (1) is the following peptide:

N(alpha)-acetyl-S N R T R I D X A N Q R A Z R M L (amide)

Where "X" is N(epsilon)-(2,4-dinitrophenyl)-lysine and "Z" is S-(fluoresceinyl)-cysteine. This peptide is a substrate for the proteolytic activity of type A botulinum neurotoxin.

Claim (2) is the following peptide:

N(alpha)-acetyl-L S E L D D R A D A L Q A X A S Q F E Z S A A K L K R -

K Y W W K N L K (amide)

Where "X" is N(epsilon)-(2,4-dinitrophenyl)-lysine and "Z" is S-(fluoresceinyl)-cysteine. This peptide is a substrate for the proteolytic activity of type B botulinum neurotoxin.

Claim 3:

Any modified peptide or protein that can serve as a substrate for the proteolytic activity of any clostridial neurotoxin, said protein or peptide having been modified to contain a signal moeity on one side of the cleavage site, and a moeity on the other side of the cleavage site that quenches or diminishes the magnitude of that signal. When the substrate is cleaved, the two diffuse away from each other and the signal increases in proportion to the amount of cleavage that has occurred. Fluorescein and dinitrophenyllysine are examples of signal and quench moeities, respectively, but others are known.

Example of an assay using a type (I) compound:

In this case, the substrate was the peptide described in claim (1) above, and the enzyme was a recombinant preparation of type A botulinum toxin catalytic domain (i.e. the "light chain"). A solution of 30 micromolar peptide was prepared in water, buffered at pH 7.0 - 7.5. Before addition of enzyme, fluorescence was measured to obtain the background or "zero-time" fluorescence. Enzyme was then added to a concentration of approximately two micrograms per ml, and the resulting increase in fluorescence due to proteolysis of the peptide was measured vs. time. Assay temperature was 21° C. In the absence of enzyme, fluorescence changed very little with time, typically less than ± 5%. Results are shown in Fig. 1.

Type (II) compounds claimed:

Claim (4) is the following peptide:

N(alpha)-fluoresceinyl-G G G S N R T R I D E A N Q R A T R M L G G G-

C(amide)

This peptide is a substrate for the proteolytic activity of type A botulinum neurotoxin.

Claim (5) is the following peptide:

N(alpha)-fluoresceinyl-G G G L S E L D D R A D A L Q A G A S Q F E S-

S A A K L K R K Y W W K N L K G G C(amide)

This peptide is a substrate for the proteolytic activity of type B botulinum neurotoxin.

Claim (6):

Any modified peptide or protein that can serve as a substrate for the proteolytic activity of any clostridial neurotoxin, said protein or peptide having been modified so that it can be attached on one side of the proteolytic cleavage site to a solid or insoluble material. The attachment point can be on either side (i.e. C-terminal or N-terminal) of the cleavage site. On the other side of the cleavage site, the substrate contains a moeity that produces a measurable signal, such as (but not limited to) a fluorescent group or a radioactive isotope. When the proteolytic activity of a clostridial neurotoxin cleaves such a substrate, the product containing the signal is released into solution. Subsequently, the amount of signal in the soluble fraction is measured. Alternatively, the amount of residual bound signal can be measured following solublization with a protease such as trypsin.

Example of assays using type (II) compounds:

In this illustration, the substrates were the peptides described in claims (4) and (5) above, and the "solid material" to which the substrates were immobilized were 96-well microtiter plates that are chemically modified to contain maleimide groups. These plates are commercially available. A solution of 15 micromolar substrate was prepared in water, buffered at pH 8.0 - 8.5, and 100 microliters were added to each well. The sulfhydryl group of cysteine in the peptide reacted with a maleimide group on the surface of the well, forming a covalent bond, thereby anchoring one end of the peptide to the well. The wells were then washed to remove unreacted peptide, and then 100 microliters of enzyme were added to each well. In this example, type A botulinum neurotoxin was added to wells containing peptide (4), while type B was added to wells containing peptide (5). For both, the concentration of enzyme was approximately two micrograms per ml. The plates were incubated at 35° C. Aliquots were removed at various times and the fluorescence in each was measured. Results are shown in Fig. 2.

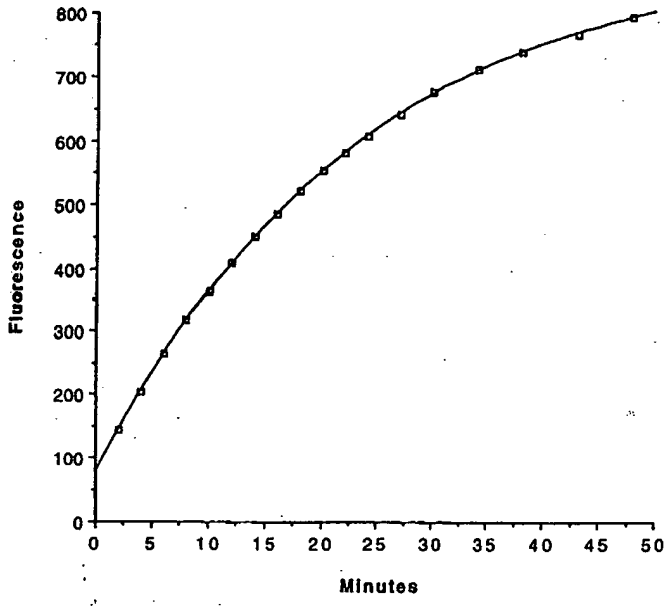

Fig. 1: Hydrolysis of peptide (1) by recombinant type A catalytic domain.

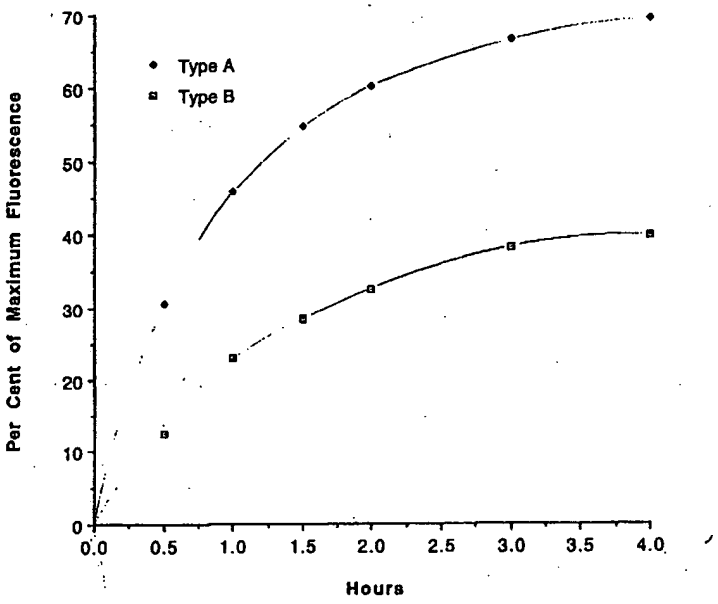

Fig. 2: Hydrolysis of immobilized substrates by types A and B botulinum neurotoxins.

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket Number: 003/204/SAP RIID 00-38X Type a plus sign {+}

INVENTOR(S)/APPLICANT(S)

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TITLE OF THE INVENTION (280 CHARACTERS MAX)

Substrates for High-Throughput Assays of Clostridial Neurotoxin Proteolytic Activities

CORRESPONDENCE ADDRESS

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STATE	ZIP CODE	COUNTRY
MD	20854	USA

ENCLOSED APPLICATION PARTS (Check all that apply)

[x] Specification Number of Pages [3] [] Small Entity Statement
[x] Drawing(s) Number of Sheets [2] [] Other (specify)

METHOD OF PAYMENT

[] A check or money order is enclosed to cover the Provisional filing fees
[x] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: **21-0380**
Provisional Filing fee amount ($) 150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
[] No.
[x] Yes, the name of the U.S. Government agency and the Government contract number are: The United States Department of the Army.

Respectfully submitted,
Signature S.A. Pratt Reg No 39,441 Date Sept 25, 2000
Typed or printed name Elizabeth Arwine for Registration No. P45,867

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